

What is claimed is:

1. A method for generating a signal indicative of the presence of a target nucleic acid sequence in a sample comprising

incubating a sample, a probe, and a structure specific nuclease,

wherein the probe has a signaling moiety, is capable of hybridizing to a target nucleic acid sequence, and does not have a secondary structure that changes upon hybridizing of the probe to the target nucleic acid sequence,

wherein the signaling moiety is inactivated when the probe is not hybridized to the target nucleic acid sequence,

wherein the signaling moiety generates a signal by the structure specific nuclease when the probe is hybridized to the target nucleic acid sequence to form a suitable substrate for the structure specific nuclease, and

wherein the generation of the signal is indicative of the presence of a target nucleic acid sequence in the sample.

2. The method of claim 1, wherein the signaling moiety of the probe comprises an indicating moiety and a regulating moiety separated by a site susceptible to the cleavage of the structure specific nuclease,  
wherein the regulating moiety inhibits the indicating moiety when the probe is not hybridized to the target nucleic acid sequence, and wherein the structure specific nuclease separates the indicating moiety from the regulating moiety when the probe is hybridized to the target nucleic acid sequence.

3. The method of claim 2, wherein the indicating moiety is a labeled moiety and the regulating moiety is a quencher moiety which quenches the labeled moiety when the probe is not hybridized to the target nucleic acid sequence.
4. The method of claim 3, wherein the labeled moiety and the regulating moiety interacts based on a resonance energy transfer.
5. The method of claim 4, wherein the resonance energy transfer is selected from the group consisting of fluorescence resonance energy transfer, luminance resonance energy transfer, phosphorescence resonance energy transfer, and bioluminescence resonance energy transfer.
6. The method of claim 1, wherein the structure specific nuclease is FEN-1 or 5' nuclease.
7. The method of claim 1, wherein the target nucleic acid sequence is a DNA sequence or an RNA sequence.
8. A method for detecting or measuring a target nucleic acid sequence in a sample comprising

incubating a sample, a probe, and a structure specific nuclease,

wherein the probe has a signaling moiety, is capable of hybridizing to a target nucleic acid sequence, and does not have a secondary structure that changes upon hybridizing of the probe to the target nucleic acid sequence,

wherein the signaling moiety is inactivated when the probe is not hybridized to the target nucleic acid sequence and the signaling moiety is activated to generate a signal by the structure specific nuclease when the probe is hybridized to the target nucleic acid sequence to form a suitable substrate for the structure specific nuclease,

detecting or measuring the amount of the signal, which is indicative of the amount of the target nucleic acid sequence present in the sample.

9. The method of claim 8, wherein the signaling moiety of the probe comprises

an indicating moiety and a regulating moiety separated by a site susceptible to the cleavage of the structure specific nuclease,

wherein the regulating moiety inhibits the indicating moiety when the probe is not hybridized to the target nucleic acid sequence, and wherein the structure specific nuclease separates the indicating moiety from the regulating moiety when the probe is hybridized to the target nucleic acid sequence.

10. The method of claim 9, wherein the indicating moiety is a labeled moiety and the regulating moiety is a quencher moiety which quenches the labeled moiety when the probe is not hybridized to the target nucleic acid sequence.

11. The method of claim 10, wherein the labeled moiety and the regulating moiety interacts based on a resonance energy transfer.

12. The method of claim 11, wherein the resonance energy transfer is selected from the group consisting of fluorescence resonance energy transfer, luminance resonance energy transfer, phosphorescence resonance energy transfer, and bioluminescence resonance energy transfer.

13. The method of claim 8, wherein the structure specific nuclease is FEN-1 or 5' nuclease.

14. The method of claim 8, wherein the target nucleic acid sequence is a DNA sequence or an RNA sequence.

15. A method for generating a signal indicative of the presence of a target nucleic acid sequence in a sample comprising

incubating a sample, a first probe, and a structure specific nuclease,

amplifying a sequence containing a target nucleic acid sequence in the sample including using a nucleic acid polymerase which does not have any substantial 5'-3' nuclease activity

wherein the first probe has a signaling moiety and is capable of hybridizing to the target nucleic acid sequence,

wherein the signaling moiety is inactivated when the first probe is not hybridized to the target nucleic acid sequence,

wherein the signaling moiety generates a signal by the structure specific nuclease when the first probe is hybridized to the target nucleic acid sequence to form a suitable substrate for the structure specific nuclease, and

wherein the generation of the signal is indicative of the presence of a target nucleic acid sequence in the sample.

16. The method of claim 15, wherein the suitable substrate for the structure specific nuclease includes a double-strand duplex, a double-strand duplex with a 5' flap, or a double-strand duplex with a 3' flap.

17. The method of claim 15 further comprises incubating a second probe capable of hybridizing to a region 5' upstream of the target nucleic acid sequence.

18. The method of claim 17 wherein the second probe is capable of hybridizing to a region 5' upstream and juxtaposed to the target nucleic acid sequence.
19. The method of claim 17 wherein the second probe is capable of hybridizing to a region 5' upstream and overlapping with the target nucleic acid sequence.
20. The method of claim 15, wherein the first probe hybridizes to the target nucleic acid sequence with a 3' flap or a 5' flap.
21. The method of claim 15, wherein the first probe contains a 3' end which is not extendable by the nucleic acid polymerase.
22. The method of claim 17, wherein the second probe is a primer used for amplifying the sequence containing the target nucleic acid sequence.
23. The method of claim 17, wherein the second probe hybridizes to the 5' upstream region of the target nucleic acid sequence with a 5' flap or a 3' flap.
24. The method of claim 15, wherein the signaling moiety of the probe comprises

an indicating moiety and a regulating moiety separated by a site susceptible to the cleavage of the structure specific nuclease,

wherein the regulating moiety inhibits the indicating moiety when the probe is not hybridized to the target nucleic acid sequence, and wherein the structure specific nuclease separates the indicating moiety from the regulating moiety when the probe is hybridized to the target nucleic acid sequence.

25. The method of claim 24, wherein the indicating moiety is a labeled moiety and the regulating moiety is a quencher moiety which quenches the labeled moiety when the probe is not hybridized to the target nucleic acid sequence.
26. The method of claim 25, wherein the labeled moiety and the regulating moiety interacts based on a resonance energy transfer.
27. The method of claim 26, wherein the resonance energy transfer is selected from the group consisting of fluorescence resonance energy transfer, luminance resonance energy transfer, phosphorescence resonance energy transfer, and bioluminescence resonance energy transfer.
28. The method of claim 15, wherein the structure specific nuclease is FEN-1 or 5' nuclease.
29. The method of claim 15, wherein the target nucleic acid sequence is a DNA sequence or an RNA sequence.
30. The method of claim 15, wherein the sequence containing the target nucleic acid sequence is amplified by isothermal amplification, thermocycling amplification, linear amplification, or exponential amplification.
31. A method for detecting or measuring the presence of a target nucleic acid sequence in a sample comprising
- incubating a sample, a first probe, and a structure specific nuclease,
- amplifying a sequence containing a target nucleic acid sequence in the sample including using a nucleic acid polymerase which does not have any substantial 5'-3' nuclease activity

wherein the first probe has a signaling moiety and is capable of hybridizing to the target nucleic acid sequence,

wherein the signaling moiety is inactivated when the first probe is not hybridized to the target nucleic acid sequence, and

wherein the signaling moiety generates a signal by the structure specific nuclease when the first probe is hybridized to the target nucleic acid sequence to form a suitable substrate for the structure specific nuclease,

detecting or measuring the amount of signal, which is indicative of the amount of the target nucleic acid sequence present in the sample.

32. The method of claim 31, wherein the suitable substrate for the structure specific nuclease includes a double-strand duplex, a double-strand duplex with a 5' flap, or a double-strand duplex with a 3' flap.
33. The method of claim 31 further comprises incubating a second probe capable of hybridizing to a region 5' upstream of the target nucleic acid sequence.
34. The method of claim 33 wherein the second probe is capable of hybridizing to a region 5' upstream and juxtaposed to the target nucleic acid sequence.
35. The method of claim 33 wherein the second probe is capable of hybridizing to a region 5' upstream and overlapping with the target nucleic acid sequence.
36. The method of claim 31, wherein the first probe hybridizes to the target nucleic acid sequence with a 3' flap or a 5' flap.
37. The method of claim 31, wherein the first probe contains a 3' end which is not extendable by the nucleic acid polymerase.

38. The method of claim 33, wherein the second probe is a primer used for amplifying the sequence containing the target nucleic acid sequence.
39. The method of claim 33, wherein the second probe hybridizes to the 5' upstream region of the target nucleic acid sequence with a 5' flap or a 3' flap.
40. The method of claim 31, wherein the signaling moiety of the probe comprises
- an indicating moiety and a regulating moiety separated by a site susceptible to the cleavage of the structure specific nuclease,
- wherein the regulating moiety inhibits the indicating moiety when the probe is not hybridized to the target nucleic acid sequence, and wherein the structure specific nuclease separates the indicating moiety from the regulating moiety when the probe is hybridized to the target nucleic acid sequence.
41. The method of claim 40, wherein the indicating moiety is a labeled moiety and the regulating moiety is a quencher moiety which quenches the labeled moiety when the probe is not hybridized to the target nucleic acid sequence.
42. The method of claim 41, wherein the labeled moiety and the regulating moiety interacts based on a resonance energy transfer.
43. The method of claim 42, wherein the resonance energy transfer is selected from the group consisting of fluorescence resonance energy transfer, luminance resonance energy transfer, phosphorescence resonance energy transfer, and bioluminescence resonance energy transfer.
44. The method of claim 31, wherein the structure specific nuclease is FEN-1 or 5' nuclease.



45. The method of claim 31, wherein the target nucleic acid sequence is a DNA sequence or an RNA sequence.

46. The method of claim 31, wherein the sequence containing the target nucleic acid sequence is amplified by isothermal amplification, thermocycling amplification, linear amplification, or exponential amplification.

47. A kit comprising a first probe and a structure specific nuclease,

wherein the first probe has a signaling moiety, is capable of hybridizing to a target nucleic acid sequence, and does not have a secondary structure that changes upon hybridizing of the first probe to the target nucleic acid sequence, and

wherein the signaling moiety is inactivated when the first probe is not hybridized to the target nucleic acid sequence and the signaling moiety is activated to generate a signal by the structure specific nuclease when the first probe is hybridized to the target nucleic acid sequence to form a suitable substrate for the structure specific nuclease.

48. The kit of claim 47, wherein the signaling moiety of the first probe comprises an indicating moiety and a regulating moiety separated by a site susceptible to the cleavage of the structure specific nuclease,

wherein the regulating moiety inhibits the indicating moiety when the first probe is not hybridized to the target nucleic acid sequence, and wherein the structure specific nuclease separates the indicating moiety from the regulating moiety when the first probe is hybridized to the target nucleic acid sequence.

49. The kit of claim 48, wherein the indicating moiety is a labeled moiety and the regulating moiety is a quencher moiety which quenches the labeled moiety when the first probe is not hybridized to the target nucleic acid sequence.
50. The kit of claim 49, wherein the labeled moiety and the regulating moiety interacts based on a resonance energy transfer.
51. The kit of claim 50, wherein the resonance energy transfer is selected from the group consisting of fluorescence resonance energy transfer, luminance resonance energy transfer, phosphorescence resonance energy transfer, and bioluminescence resonance energy transfer.
52. The kit of claim 47, wherein the structure specific nuclease is FEN-1 or 5' nuclease.
53. The kit of claim 47, wherein the target nucleic acid sequence is a DNA sequence or an RNA sequence.
54. A kit comprising a first probe, a structure specific nuclease, and a nucleic acid polymerase which does not have any substantial 5'-3' nuclease activity,

wherein the first probe has a signaling moiety and is capable of hybridizing to a target nucleic acid sequence,

wherein the nucleic acid polymerase is capable of amplifying a sequence containing the target nucleic acid sequence,

wherein the signaling moiety is inactivated when the first probe is not hybridized to the target nucleic acid sequence, and

wherein the signaling moiety generates a signal by the structure specific nuclease when the first probe is hybridized to the target nucleic acid sequence to form a suitable substrate for the structure specific nuclease.

55. The kit of claim 54, wherein the suitable substrate for the structure specific nuclease includes a double-strand duplex, a double-strand duplex with a 5' flap, or a double-strand duplex with a 3' flap.
56. The kit of claim 54 further comprises a second probe capable of hybridizing to a region 5' upstream of the target nucleic acid sequence.
57. The kit of claim 56 wherein the second probe is capable of hybridizing to a region 5' upstream and juxtaposed to the target nucleic acid sequence.
58. The kit of claim 56 wherein the second probe is capable of hybridizing to a region 5' upstream and overlapping with the target nucleic acid sequence.
59. The kit of claim 54, wherein the first probe is capable of hybridizing to the target nucleic acid sequence with a 3' flap or a 5' flap.
60. The kit of claim 54, wherein the first probe contains a 3' end which is not extendable by the nucleic acid polymerase.
61. The kit of claim 56, wherein the second probe is a primer used for amplifying the sequence containing the target nucleic acid sequence.
62. The kit of claim 56, wherein the second probe is capable of hybridizing to the 5' upstream region of the target nucleic acid sequence with a 5' flap or a 3' flap.

63. The kit of claim 54, wherein the signaling moiety of the probe comprises
- an indicating moiety and a regulating moiety separated by a site susceptible to the cleavage of the structure specific nuclease,
- wherein the regulating moiety inhibits the indicating moiety when the probe is not hybridized to the target nucleic acid sequence, and wherein the structure specific nuclease separates the indicating moiety from the regulating moiety when the probe is hybridized to the target nucleic acid sequence.
64. The kit of claim 63, wherein the indicating moiety is a labeled moiety and the regulating moiety is a quencher moiety which quenches the labeled moiety when the probe is not hybridized to the target nucleic acid sequence.
65. The kit of claim 64, wherein the labeled moiety and the regulating moiety interacts based on a resonance energy transfer.
66. The kit of claim 65, wherein the resonance energy transfer is selected from the group consisting of fluorescence resonance energy transfer, luminance resonance energy transfer, phosphorescence resonance energy transfer, and bioluminescence resonance energy transfer.
67. The kit of claim 54, wherein the structure specific nuclease is FEN-1 or 5' nuclease.
68. The kit of claim 54, wherein the target nucleic acid sequence is a DNA sequence or an RNA sequence.
69. The kit of claim 54, wherein the nucleic acid polymerase and the structure specific nuclease is a recombinant protein.

70. The kit of claim 54, wherein the nucleic acid polymerase and the structure specific nuclease is provided as a single recombinant protein.
71. The kit of claim 54 further comprises at least one amplification primer useful for amplifying the sequence containing the target nucleic acid sequence.
72. The kit of claim 54 further comprises a forward amplification primer and a reverse amplification primer useful for amplifying the sequence containing the target nucleic acid sequence.